

Resistant Starch: Promise for Improving Human Health^{1,2}

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ABSTRACT

Ongoing research to develop digestion-resistant starch for human health promotion integrates the disciplines of starch chemistry, agronomy, analytical chemistry, food science, nutrition, pathology, and microbiology. The objectives of this research include identifying components of starch structure that confer digestion resistance, developing novel plants and starches, and modifying foods to incorporate these starches. Furthermore, recent and ongoing studies address the impact of digestion-resistant starches on the prevention and control of chronic human diseases, including diabetes, colon cancer, and obesity. This review provides a transdisciplinary overview of this field, including a description of types of resistant starches; factors in plants that affect digestion resistance; methods for starch analysis; challenges in developing food products with resistant starches; mammalian intestinal and gut bacterial metabolism; potential effects on gut microbiota; and impacts and mechanisms for the prevention and control of colon cancer, diabetes, and obesity. Although this has been an active area of research and considerable progress has been made, many questions regarding how to best use digestion-resistant starches in human diets for disease prevention must be answered before the full potential of resistant starches can be realized. *Adv. Nutr.* 4: 587–601, 2013.

Introduction

Growing evidence shows that many of the chronic health conditions in developed countries could be prevented or moderated by dietary changes. The most common starchy foods in the United States diet, including white bread, cakes, and noodles, consist of a large percentage of highly digestible starch. There is concern that such rapidly digested starches may contribute to chronic disease in people and animals and, because of this problem, starches that are resistant to digestive enzymes have been the focus of a growing research emphasis. Such starches, termed resistant starches (1), have been extensively reviewed in general (2) and reviewed from the standpoint of their health properties (3); increasing their content in food components (4); their health and functional properties as a food ingredient (5); and their role in gut health, potentially through butyrate production (6). The estimated daily intake of resistant starch by Americans is ~5 g

per day, much less than the minimum of 6 g of resistant starch per meal recommended for health benefits (7).

This review summarizes the types of digestion-resistant starches, the complexity associated with the analysis of different types of resistant starch, and the current status of resistant starches in foods. This review addresses how, after ingestion, normal food starch is rapidly digested and absorbed as glucose, potentiating a hyperglycemic response and triggering insulin secretion and tissue-specific intracellular uptake of glucose that can then result in hypoglycemia. Repetition of this hyper- and hypoglycemic cycle appears to result in insulin resistance and type 2 diabetes, thereby contributing to obesity. In contrast, enzyme-resistant starches pass through the upper digestive tract to the colon, where they are fermented by bacteria, producing important metabolites, including SCFAs. These metabolites appear to have important biological effects, including reduction of colon cancer precursors, systemic regulation of macronutrient metabolism, and altered secretion of hormones, which can lead to improved physical and mental health.

Ongoing research by the authors of this review has provided important new evidence of the health benefits of resistant starches, showing that resistant starches with different characteristics cause different changes to the bacteria that

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TABLE 1 Types of resistant starches¹

| Designation | Description | Example | Reference |
|-------------|--|--|-----------|
| RSI | Physically inaccessible starch | Coarsely ground or whole-kernel grains | (1) |
| RSII | Granular starch with the B- or C-polymorph | High-amylose maize starch, raw potato, raw banana starch | (1) |
| RSIII | Retrograded starch | Cooked and cooled starchy foods | (24) |
| RSIV | Chemically modified starches | Cross-linked starch and octenyl succinate starch | (25) |
| RSV | Amylose-lipid complex | Stearic acid-complexed high-amylose starch | (31) |

¹ RSI, type I resistant starch; (RS); RSII, type II resistant starch; RSIII, type III resistant starch; RSIV, type IV resistant starch; RSV; type V resistant starch.

colonize the colon (i.e., microbiota) (8–11) and that resistant starches can prevent or attenuate many of the parameters characteristic of vitamin D deficiency associated with type 1 diabetes (12).

Types of Resistant Starches

Resistant starch is defined as a portion of starch that cannot be digested by amylases in the small intestine and passes to the colon to be fermented by microbiota (13). Englyst et al. (1) proposed a classification system based on starch digestive rate. This system divides starches into rapidly digestible starches, slowly digestible starches, and resistant starches based on the results of in vitro digestion. There are currently 5 types of resistant starch (Table 1). Substantial research has been conducted on each of the 5 types of resistant starch, and they are briefly summarized next.

Type I. Starch is synthesized in the endosperm of cereal grains or seeds, and starch granules are surrounded by protein matrix and cell wall material. These physical structures hinder the digestibility of starch and reduce the glycemic response (14). When cooked as whole kernels or coarsely ground seeds, the thick cell wall of legume seeds and the protein matrix in cereal grains prevent water penetration into the starch in the matrix. Therefore, the starch does not have adequate moisture to readily gelatinize and swell. Without proper swelling to expose the starch molecules, the starch is not readily susceptible to enzymatic hydrolysis. The cell wall material and the protein matrix also provide a physical barrier, preventing enzymes from reaching and hydrolyzing the starch. Examples of type I resistant starch (RSI)⁹-containing foods are breads made with whole or coarsely ground kernels of grains (15) and pasta made with durum wheat by extrusion (16). Durum wheat has a high protein content and hard texture and is used for making semolina with coarse particles. Consequently, the postprandial glycemic response is substantially lower after ingesting semolina pasta compared with white bread. Residual starch that is not digested in the small intestine passes into the colon as RSI.

Type II. Uncooked potato starch, green banana starch, ginkgo starch, and high-amylose maize starch, which display the B- or C-type polymorph, are highly resistant to enzymatic hydrolysis (17) and are examples of type II resistant

starch (RSII). However, after cooking, most of the starch, such as that in baked potato and cooked banana, becomes highly digestible as a result of starch gelatinization and loss of the B- and C-type crystallites. An exception is high-amylose starch produced by mutation of the *amylose-extender* (*ae*) gene and the gene encoding starch branching-enzyme I, which has substantially longer branch chains of intermediate components and a larger proportion of amylose (18–20). Thus, this starch displays a high gelatinization temperature, above the boiling point of water. After boiling or cooking at a temperature below its gelatinization temperature, this type of starch retains its crystalline structure and remains resistant to enzymatic hydrolysis.

Type III. Type III resistant starch (RSIII) is retrograded amylose and starch (21–23). Because amylose molecules have linear structures, they have a great tendency to form double helices, particularly near refrigeration temperatures (4–5°C) and with adequate moisture content. Retrograded amylose has high gelatinization temperatures, up to 170°C, and cannot be dissociated by cooking. The gelatinization temperature of retrograded amylose, however, decreases with shortening of the amylose chain length. After starchy foods are stored, particularly in a refrigerator, amylose molecules and long branch chains of amylopectin form double helices and lose their water-binding capacity. The double helices of starch molecules do not fit into the enzymatic binding site of amylase, thus they cannot be hydrolyzed by this enzyme.

Type IV. Type IV resistant starch (RSIV) is a chemically modified starch, formed either by cross-linking (24,25) or by adding chemical derivatives (26). Starch with a high level of cross-linking loses the ability to swell during cooking. Consequently, the highly cross-linked starch remains in a granular form after cooking, with little enzymatic susceptibility, and cannot be hydrolyzed by amylases or fermented by microbes. Adding a chemical derivative to starch, such as octenyl succinic groups (27) or acyl groups (28), changes the structure of the starch and partially restricts the enzymatic hydrolysis of the starch molecule, resulting in resistant starch. A region of the starch without the derivative can be hydrolyzed by bacterial amylases and fermented to produce short-chain fatty acids.

Type V. When starch interacts with lipids, amylose and long branch chains of amylopectin form single-helical complexes with fatty acids and fatty alcohols (21,29). When the linear starch chain is in a helical-complex structure with the

⁹ Abbreviations used: 25D3, 25-hydroxycholecalciferol; DBP, vitamin D-binding protein; GI, gastrointestinal; rRNA, ribosomal RNA; RSI, type I resistant starch; RSII, type II resistant starch; RSIII, type III resistant starch; RSIV, type IV resistant starch; RSV, type V resistant starch.

complexed fatty acid in the cavity of the helix, starch binding and cleavage by amylase are prevented. In addition, the amylose-lipid complex also entangles amylopectin molecules, restricting the swelling of starch granules and enzyme hydrolysis (30,31). Because the amylose-lipid complex formation is an instant reaction and the complex can reform after cooking, type V resistant starch (RSV) is considered thermally stable.

It is important to recognize that starch digestibility is influenced by nonstarch components in the digest, the structure of the starch, and starch processing before digestion. The digestibility of a given starch sample is never due to a single factor as classification systems suggest; rather, the extrinsic factor with the greatest influence on digestibility is generally used to classify the starch.

The botanical role of starch is to provide plants with a stable reserve of glucose for metabolism. The digestibility of starch is an important parameter in meeting this role. The glucose reserves must be stored in a structure that is readily available to the plant, yet able to survive for long periods of time in storage organs such as seeds or tubers. The structure of starch is complex and varies widely; however, the single structural aspect with the greatest influence on digestibility is the degree and type of crystallinity within the granule. Starch with long, linear chains has a greater tendency to form crystalline structures than starch with short, highly branched chains. Because the amylose component of starch is less branched than amylopectin, high-amylose starch tends to be more resistant to digestion than low-amylose starch.

Sources of Variation in Botanical Resistant Starch

Environment. The field production environment has an impact on starch thermal properties (32) and starch digestibility (33). This effect may be a consequence of environmental conditions such as temperature altering the activity of starch biosynthetic enzymes (34). Environmental variation in resistant starch content is difficult to predict and control; therefore, it has not been used as a tool for increasing resistant starch levels.

Natural genetic variation. Starch structure varies with botanical source. Additional genetic variation occurs within botanical sources because of allelic variation in starch biosynthesis genes. In commercial maize varieties, for example, there is little variation in resistant starch levels, but exotic germplasm contains substantial variation in resistant starch content (33,35).

Mutations. In maize the *ae* locus encodes starch branching-enzyme 2b (36). Mutations at this locus result in starch with higher apparent amylose content (37). Campbell et al. (38) identified exotic germplasm that in combination with *ae* produces starch that is more resistant to digestion than normal *ae* germplasm. Genetic studies suggest that the modifier of *ae* encodes starch branching-enzyme I (39). Taken together, these studies suggest that the modification of starch

branching enzymes is an effective approach to altering resistant starch content. In barley, the *sex6* locus encodes starch synthase IIa and a mutation at this locus has 20% of normal amylopectin content (40). In a study of human participants, diets containing this barley improved several measures of gut health compared with diets based on refined starch (41).

Biotechnology. Understanding the molecular mechanisms controlling starch structure as it relates to digestibility has allowed genetic engineering approaches to production of starch with increased resistance to digestion. Several examples show that suppression of starch branching-enzyme genes results in increased amylose content in many species (42–46). The ultimate use of this approach would be to eliminate all starch branching-enzyme activity. Toward this goal, all 3 starch branching enzymes in barley were suppressed using RNA interference, resulting in starch with 100% amylose that was highly resistant to digestion (47).

Methods of Starch Analysis

Dietary fiber is defined by the American Association of Cereal Chemists (48) as “the edible part of plant foods or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the small intestine.” Resistant starch is similarly functionally defined as “the starch fraction that escapes digestion in the small intestine of healthy humans.” Although in vivo analysis of starch digestibility in humans may be considered ideal, in vitro analytical methods for resistant starch contents of foods deriving from dietary fiber analysis methods have been introduced and standardized. Analysis of dietary fiber/ and resistant starch content in foods is based on hydrolysis of available carbohydrate in a sample, which may be accompanied by proteolysis to remove the protein surrounding starch and make the starch susceptible to amylase hydrolysis. Common analytical methods for dietary fiber content of foods include AOAC Codex methods 991.43 (Total, Soluble and Insoluble Dietary Fiber in Foods, 1994), 2001.03 (Dietary Fiber Containing Supplemented Resistant Maltodextrin, 2004), 2009.01 (Total Dietary Fiber in Foods, 2009), and 2011.25 (Insoluble, Soluble and Total Dietary Fiber in Foods, 2011) (49) and the Englyst method for dietary fiber analysis (50). Methods specifically designed for determination of resistant starch content of foods include AOAC method 2002.02 (Resistant Starch in Starch and Plant Materials, 2002) and the Englyst method for resistant starch analysis (1,49).

A brief summary of selected methods is presented here. AOAC method 991.43 is used for food labeling in the United States and European Union and measures total dietary fiber as the filtrate residue of a food sample after digestion with a thermostable α -amylase from *Bacillus licheniformis*. This method is rapid but is appropriate for determination of thermostable resistant starch only, because some types of resistant starch, such as raw potato starch granules (RSII), may be destroyed during sample processing. AOAC method 2002.02 is a glucogenic method intended for use with raw starch samples

and gives a direct measure of resistant starch in the intact starch granule. Samples are hydrolyzed for 16 h using purified porcine pancreatic α -amylase and amyloglucosidase from *Aspergillus niger*. The residue collected after digestion is dried and dispersed in potassium hydroxide solution and digested using amyloglucosidase to give the resistant starch fraction. Total starch content of the sample is taken as the sum of digestible starch and resistant starch fractions. However, use of purified porcine pancreatic α -amylase and the prolonged 16-h digestion period may not accurately mimic conditions of human digestion. In addition, the procedure is lengthy and has numerous opportunities for human error during decanting of supernatants, rendering it impractical for rapid analysis of samples containing resistant starch. The Englyst method for resistant starch analysis (1) is intended to mimic the process of human digestion as accurately as can be achieved in vitro. This method uses an aqueous extract of crude porcine pancreatin mixed with amyloglucosidase for hydrolysis of starch up to 2 h. Samples generally are used as is for whole foods or are boiled for raw foods before hydrolysis. Aliquots are withdrawn after 20 and 120 min for determination of rapidly digestible starch and slowly digestible starch fractions, respectively; resistant starch is taken as the remaining undigested starch out of the total starch content of the sample. Some variations of this method use predigestion with trypsin, HCl, or both for better simulation of physiological digestion.

Because dietary fiber and resistant starch, by definition, survive transit in the small intestine, direct measurement of starch digestibility in isolated starches and whole foods in patients with an ileostomy who are free of active disease may be considered a true measure of resistant starch and dietary fiber content in foods. Physiological measurements used as an indirect measure of starch digestibility in vivo include postprandial serum glycemia, breath $^{13}\text{CO}_2$ measurements (51), and 2-h postprandial thermogenesis (52).

Diets supplemented with native raw potato and banana starches, representing RSII, administered to patients with an ileostomy have been investigated. Faisant and others (53) investigated digestibility of green banana starch in human ileostomates and found that a fraction composed mainly of intact granular starch and resistant oligosaccharides comprising 83.7% of the original starch weight reached the ileal terminus. This value was higher than resistant starch content in banana starch determined through in vitro analyses using a modified Englyst method (about 70% resistant starch). This difference was attributed to a combination of factors, including potential differences in banana ripeness, potential failure of the in vitro analyses to account for resistant oligosaccharides, and kinetic motion of the stomach and small intestine that is not easily replicated by in vitro methods. Cooked potatoes administered to patients with an ileostomy showed only 0.82% resistant starch in freshly cooked potatoes, whereas potatoes that had been allowed to cool showed 1.60% resistant starch when analyzed using an Englyst method procedure with an acid hydrolysis step (54). Resistant starch contents of these 2 different meals in ileal effluent were 0.54% and 1.48%, respectively, showing good

agreement with the in vitro method results. The differences between freshly cooked and cooled potato meals were attributed to retrogradation of amylose and long amylopectin chains characteristic of potato starches.

Vonk and colleagues (51) used breath $^{13}\text{CO}_2$ measurements as an indicator of gut fermentation for up to 6 h postprandium to compare in vivo digestibility of normal corn starch, Hylon VII (high-amylose corn starch; Ingredion), and Novelose 330 (a commercial RSIII prepared from retrograded high-amylose corn starch; Ingredion). Breath $^{13}\text{CO}_2$ measurements were in good agreement with general trends observed in starch contents of ileal effluents of individuals fed whole diets containing similar amounts of normal and high-amylose corn starches (55,56).

Animal models used to examine digestibility of resistant starches include rats (57), pigs (58,59), and rabbits (60). Digestibility of uncooked starches in animal models and digestibility in humans must be compared with care because of differing physiologies and, less importantly, differential raw starch-degrading activity of animal peptic amylases compared with those of humans. For example, porcine pancreatic α -amylase is commonly used as a surrogate for human pancreatic α -amylase because of its similarity to the human enzyme (61,62). Although the rat enzyme shares 83% sequence homology with human pancreatic α -amylase (63), it has been shown to have higher initial starch-degrading activity than porcine pancreatic α -amylase on starches of raw, normal maize; green banana; and potato (64). Rather than measuring digestibility of starch content per se, animal models generally have been used to determine effects of resistant starch intake on existing health problems in humans, such as diabetes (65), obesity (66), and cancers of the bowel (67). Because animals must generally be killed to determine physiological effects of resistant starch, a nondestructive means for determining starch digestibility in animal models is desirable. Recently, Anderson et al. (68) described an empirical method to determine starch in rat cecal contents using Fourier transform infrared photoacoustic spectroscopy through partial least squares calibration. Reference starch content values for calibrating the partial least squares model were determined using the Megazyme total starch kit method. The model correlated cecal content spectra with starch content with a cross-validation correlation coefficient (R^2) of 0.997. Empirical methods such as Fourier transform infrared photoacoustic spectroscopy may be an inexpensive, rapid method for determining resistant starch contents in feces in the context of animal studies using similar diets.

Challenges in Food Product Development

The current intake of resistant starch by Americans is lower than recommended for providing health benefits, emphasizing the need for Americans to increase the resistant starch content of their diets (7). Foods such as potatoes, rice, pasta, breakfast cereals, and bread are low in resistant starch (<2.5%, dry matter basis). Cooked legumes, peas, and cooked and cooled starchy foods are high in resistant starch (5.0–15%,

dry matter basis) (69). Most foods in a typical Western diet contain highly digestible starches and have a high glycemic index. **Table 2** compares the resistant starch content and glycemic index for several starch-based foods (7,70). Because of the health benefits related to foods with increased resistant starch and decreased glycemic index, there is a growing interest in developing foods with increased resistant starch contents (2,7).

The structural characteristics of resistant and normal starches are different and relevant to the development of resistant starch products (71). The characteristics of the different types of resistant starch reflect the effect of processing, starch granule characteristics, and gelatinization on the starch structure. Heating starch in the presence of adequate water contributes to starch gelatinization and an increase in digestibility. With cooling, starches with high amylose contents retrograde and form crystalline regions not accessible to enzymatic hydrolysis (72). However, swelling and gelatinization of high-amylose starches are reduced compared with normal starches as a result of the higher degree of crystallinity (71).

Because the different types of resistant starch differ in their composition and structure, the effects of processing on each type of resistant starch need to be considered individually. With RSI, digestive enzymes are unable to hydrolyze the starch because of an inability to penetrate the cell wall materials. Therefore, grinding or homogenization of the grains can break down the cell structures and decrease the resistant starch content. RSII is found in raw starchy foods, and the resistant starch content would be expected to decrease during processing that results in starch gelatinization. On the other hand, RSIII is formed when starches gelatinize and recrystallize, thus resistant starch contents could increase when those foods received further heat treatment (7).

Apart from intrinsic properties of starch affecting the formation of resistant starch (e.g., crystallinity, the ratio of amylose to amylopectin, granular structure), processing factors affect resistant starch content and formation in processed food. Baking, pasta production, extrusion cooking, autoclaving, and other processes are known to affect both starch gelatinization and retrogradation and influence the yield of resistant starch in finished food (2). Some studies have reported on the influence of extrusion on the resistant starch content of corn flakes and puff breakfast cereals and snacks. For example, the formation of RSIII (mainly retrograded amylose) in hull-less barley flours was generally influenced, but not greatly, by extrusion cooking at different temperatures, moisture contents, and screw speeds (73). Huth et al. (69) reported that extrusion conditions optimizing starch gelatinization, depolymerization, retrogradation, and recrystallization contribute to higher contents of resistant starch in extruded barley. Using a single-screw extruder, both temperature and moisture content were optimized to maximize starch gelatinization, with feed moisture at 20% and a mass temperature of 150°C resulting in the highest resistant starch content. During extrusion, the shear stress of the screw results in depolymerization of starch, which further contributes to resistant starch formation. As the extruded material leaves the extruder, resistant starch formation

TABLE 2 Comparison of resistant starch content and glycemic index for commonly consumed starchy foods¹

| | Resistant starch | Glycemic index |
|---------------------------|------------------|----------------|
| | g/100 g | |
| Grain and cereal products | | |
| Buckwheat | 1.8 | 51 |
| Bread (white) | 1.2 | 69 |
| Bread (whole meal) | 1.0 | 72 |
| Millet | 1.7 | 71 |
| Rice (brown) | 1.7 | 66 |
| Rice (white) | 1.2 | 72 |
| Spaghetti (whole meal) | 1.4 | 42 |
| Spaghetti (white) | 1.1 | 50 |
| Breakfast cereals | | |
| All-Bran (Kellogg's) | 0.7 | 51 |
| Cornflakes | 3.2 | 80 |
| Muesli | 3.3 | 66 |
| Porridge oats | 0.2 | 49 |
| Shredded wheat | 1.2 | 67 |
| Wheatabix | 0.1 | 75 |
| Vegetables | | |
| Broad beans | 1.2 | 79 |
| Potatoes (white) | 1.3 | 80 |
| Potatoes (sweet) | 0.7 | 48 |
| Sweetcorn | 0.3 | 59 |
| Yam | 1.5 | 1.5 |
| Legumes | | |
| Beans (baked) | 1.2 | 40 |
| Beans (kidney) | 2.0 | 29 |
| Peas (chick) | 2.6 | 36 |
| Lentils | 3.4 | 29 |

¹ Resistant starch data from (7); glycemic index data from (70).

continues through retrogradation and recrystallization of the starch (69).

Use of resistant starch in baked products is limited because of adverse quality effects of resistant starch on texture, softness, gas cell size, and gluten network formation (74–76). Studies have been reported on the natural formation of RSIII and its use as an ingredient in food products (77,78). Addition of RSIII resulted in decreased pliability, rollability, and cohesiveness in flour tortillas. High concentrations of RSIII led to reduced structural integrity and therefore decreased quality of the product (74,79). The addition of 5–20% resistant starch to muffins contributed to overall decreased quality, with chewiness, cohesiveness, and volume affected (80,81). Resistant starch type has had an impact on acceptability and textural quality when incorporated into muffins. Although the incorporation of either RSII or RSIII decreased muffin quality, the quality of muffins with RSII was more acceptable than muffins with RSIII (78).

The substitution of 20% RSV with added vital wheat gluten for wheat flour resulted in bread with 11.5% resistant starch that was comparable to the control. Substitutions up to 50% resulted in increased resistance to gelatinization and decreased gluten network formation, contributing to decreased loaf volume, increased density, and decreased consumer acceptability. The addition of dough conditioners, flavor maskers, and colorants improved the sensory attributes of the breads (MO Reed, TD Boylston, and J Jane, unpublished results).

Further research is necessary to understand the effects of processing on the content of resistant starch in a range of food products. Depending on the type of resistant starch in these food products, the use of various food additives may be necessary to produce foods with acceptable quality attributes. The development of food products with increased resistant starch contents and acceptable quality attributes is imperative to provide foods with increased health benefits to consumers.

Gut Bacterial Metabolism of Digestion-Resistant Starch

Bacteria in the lower intestines of humans may be exposed to as much as 20 g of resistant starch per day (82). The fermentation products of resistant starch by gut bacteria include gases (methane, hydrogen, carbon dioxide) and SCFAs (acetate, propionate, butyrate, and valerate). Much lesser amounts of organic acids (lactate, succinate, and formate), branched SCFAs (isobutyrate and isovalerate), and alcohols (methanol and ethanol) are also produced. Starch degradation is a cooperative process in the lower gut, generalized as 1) degradation of starch polymers into glucose; 2) glycolysis with SCFA or other organic acids as end products; and 3) methane production by methanogenic *Archaea* spp. from formate, hydrogen gas, and carbon dioxide products of bacterial metabolism of resistant starch. These processes involve several bacterial groups, as described next.

Amylolytic gut bacteria. Gut bacterial amylase-mediated starch breakdown includes α -amylase for α -1,4 linkage, type I pullulanase for α -1,6 linkage, and amylopullulanases for both α -1,4 and α -1,6 linkages (83). Three major phyla, *Firmicutes*, *Bacteroidetes*, and *Actinobacterium*, which account for 95% of total mammalian gut bacteria, are involved in starch fermentation. Macfarlane et al. (84) used peptone-yeast agar plates to screen soluble starch-hydrolyzing strains, noted by clearing zones around colonies. From 120 amylolytic colonies randomly selected from fecal samples of 6 human participants, 58% were identified as *Bifidobacterium* spp., with lactate and acetate as major products. About 18% of starch-hydrolyzing fecal isolates were identified as *Bacteroides* spp., with acetate and propionate as major products, and another 10% were identified as *Fusobacterium* and *Butyrivibrio*, with butyrate as the major product. Wang et al. (85) screened 38 human colonic bacteria strains with this technique (84) but used autoclaved starch granules to mimic food preparation. Although *Bifidobacterium* spp.; *Bacteroides* spp.; *Fusobacterium* spp.; and strains from *Eubacterium*, *Clostridium*, *Streptococcus*, and *Propionibacterium* all had amylase activity to utilize high-amylopectin and soluble starch, only *Bifidobacterium* spp. and *Clostridium butyricum* could efficiently utilize high-amylose starch granules. These species would be of particular importance to gut bacterial fermentation of high-amylose starch (i.e., RSII).

Bacterial binding to starch granules is probably important for bacterial starch fermentation in the lower intestine. Two structures have been identified for starch binding: the

cellulosome in *Ruminococcus flavefaciens* for insoluble substrates (86) and outer membrane protein complex in *Bacteroides thetaiotaomicron* for soluble substrates, including starch-utilization-structure (*sus*) gene clusters that bind to and hydrolyze starch (87). It is unknown whether other *Ruminococci* have cellulosomes, but *Ruminococcus bromii*, enriched by resistant starch in vivo and in vitro (88), is closely related to *R. flavefaciens*.

The starch-binding ability in other Gram-positive bacteria might be mediated by cell-associated α -amylase. Cell-associated amylases were identified in *Butyrivibrio fibrisolvens*, *Roseburia inulinivorans*, and *Roseburia intestinalis*, all butyrate-producing bacteria (11). Cosedimentation was used to examine the affinity of 19 *Bifidobacterium* strains with high-amylose starch in vitro (12). Two amylolytic strains, *Bifidobacterium pseudolongum* (American Type Culture Collection 25526) and *Bifidobacterium adolescentis* (Technical Research Centre of Finland E-001561), had the highest affinity and specificity for α -1,4-linked glucose sugars. Starch binding could be inhibited by pancreatin and low pH (<3), indicating that cell surface proteins were involved in starch attachment. Strong attachment by *Bifidobacterium* spp. to starch granules might partly explain the enrichment of these species by dietary resistant starch.

Butyrogenic bacteria. Barcenilla et al. (89) described isolation of butyrogenic bacteria in 2 fecal samples each from 1 infant, 1 adult omnivore, and 1 adult vegetarian. Bacteria from fresh fecal suspension was grown on M2 medium supplemented with glucose, soluble starch, and cellobiose, and butyrate production >2 mM in this medium was used as the criterion for designating butyrate-producing bacteria (23% of the >300 isolates tested showed this ability). Based on full-length 16S ribosomal RNA (rRNA) sequences, 80% of butyrate-producing isolates fell within the XIVa bacterial cluster, with the most abundant group (42%) related to *Eubacterium rectale*, *Eubacterium ramulus*, and *Roseburia cecicola*. Many members from cluster XIVa (or *Roseburia cecoides*/*E. rectale* group) and cluster IV (*Clostridium leptum* group) (families *Lachnospiraceae* and *Ruminococcaceae*, respectively) are frequently detected in human fecal microbiota, as summarized by Pryde et al. (90).

Two mechanisms for butyrate production involving butyrate kinase and butyryl-CoA:acetate-CoA transferase have been inferred (91). After hydrolysis of glucose through the Embden-Meyerhof-Parnas pathway, pyruvate is the major precursor of butyrate, with further conversion into acetyl-CoA, lactate, and succinate by gut microorganisms. Acetyl-CoA can be used for butyrate synthesis by butyrate kinase (92) or converted into acetate. Acetate can also be utilized by butyryl-CoA:acetate-CoA transferase as a CoA acceptor in bacteria, such as *Roseburia intestinalis* (93). A third route has been implicated recently by Belenguer et al. (94) who cocultured lactate-producing bacteria *B. adolescentis* with starch-utilizing bacteria *Eubacterium hallii* and *Anaerostipes caccae* in yeast extract-casitone-fatty acid medium supplemented with soluble potato starch. From tracing

the (1-¹³C)-labeled acetate or (U-¹³C)-labeled lactate, butyrate was converted directly from lactic acid without involvement of exogenous acetate.

The distribution of genes encoding butyrate kinase (*buk*) and butyryl-CoA:acetate-CoA transferase (*ptb*) in gut bacteria were largely unknown until the study of Louis et al. (95). They obtained 38 butyrate-producing isolates from 4 participants and identified the genes using PCR. They found that only 4 out of 38 butyrate-producing strains contained butyrate kinase genes, whereas the butyryl-CoA:acetate-CoA transferase gene was detected in all isolated bacteria, consistent with another report that 50% of butyrate-producing bacterial isolates showed significant correlation between butyrate production and acetate disappearance ($r^2 = 0.6$) (89). Sequences (1718) were obtained with degenerate primers to amplify butyryl-CoA:acetate-CoA transferase genes from fecal samples from 10 healthy humans (99). Thirty-two butyryl-CoA:acetate-CoA transferase-related sequences (cutoff > 98% similarity) were found, 4 from *E. rectale*, *Roseburia faecis*, *E. hallii*, and an unnamed cultured species SS2/1 that was highly abundant in the human gut.

In addition to the varying abundance of bacterial species or key functional genes in the gut, butyrate-producing ability may vary among bacterial species. Louis et al. (95) showed varied activity of butyrate kinase and butyryl-CoA:acetate-CoA transferase among strains and species of butyrate-producing isolates. Metagenomic analysis revealed butyrate kinase-related genes as one of the most enriched Clusters of Orthologous Groups in fecal bacteria from 2 human participants, whereas butyryl-CoA:acetate-CoA transferase was not enriched (97). Thus, investigators using differing approaches have implicated either bacterial butyrate kinase or butyryl-CoA:acetate-CoA transferase to predominate in butyrate-forming gut bacterial pathways, based on a very limited number of fecal samples. Other genes regulating butyrate production have not been identified. In addition, although some *Bacteroides* spp. and *Clostridium* spp. are known to produce propionate, its production pathway and the regulation of its production in bacteria are unknown. Detailed functional analysis of bacterial SCFA-producing activity is antipated with transcriptomics.

Methanogenic Archaea. The production of methane, which can be considered as the final end product consuming hydrogen and carbon dioxide, is another fermentation outcome. The distribution of methanogenic *Archaea* in human fecal bacterial populations is an example of interindividual variability. The proportion of methane producers in the population varied from 24% in Asians to 48% in Caucasians by measuring breath hydrogen after lactulose intake (98).

Methanogenic *Archaea* might affect the fermentation capacity of individuals. The abundance of methanogenic *Archaea* was negatively related to fecal butyrate concentration ($r = -0.729$, $P < 0.05$, $n = 8$) but not to other SCFAs, with PCR-denaturing gradient gel electrophoresis analysis using methanogen-specific primers coupled with real-time PCR (99). This study confirmed results from human and

rat in vitro fecal fermentation, showing that individuals whose feces produced greater amounts of methane also produced lesser amounts of butyrate (100). Although genome sequences of 2 methanogens are available, their physiological and health importance is still unknown. With the great range of possible competition and cross-feeding among gut bacterial species, further studies are needed to identify the role that methanogenic *Archaea* play in resistant starch fermentation.

In summary, the utilization of resistant starch is cooperative and redundant in the gut. The identification of bacteria or bacterial functions related to resistant starch fermentation is important for predicting health outcomes of ingesting resistant starch. Progress has been made in identifying the gut bacteria related to resistant starch fermentation and in characterizing the metabolic mechanisms of bacterial resistant starch fermentation, which are crucial for understanding mechanisms and conditions under which digestion-resistant starches may help to prevent diseases.

Resistant Starch and Gut Microbiota

It is often recognized that the mammalian gastrointestinal (GI) tract is home to more bacterial cells than comprise the entire host. It is also well established that GI microbiota make important contributions to the health of the host, including immune system development, nutritional acquisition, and protection against infection (101). In recent years, new evidence has greatly increased our understanding of microbiota impacts on host health. For example, an altered microbiota (dysbiosis) has been associated with human diseases, such as diabetes, obesity, inflammatory bowel diseases, and colorectal cancer (101). GI microbiota have recently been shown to contribute to neurological diseases and influence host behavior (102). These insights have led to a heightened interest in the role of the diet in modulation of GI microbiota as a means to improve host health (91,103,104) and a new awareness of how diet can contribute to disease (101,105).

It has long been known that diet influences the microbial communities of the GI tract. Diet-induced changes in these microbiota can have beneficial effects on the health of the host through the breakdown of dietary fibers and production of SCFAs, which are an important source of energy for the host and perform important immune modulatory roles (106,107). Although studies to understand how different classes of resistant starch affect microbiota are limited, it is clear that high-fiber diets greatly affect the composition of mammalian microbiota (9,10). Microbiota also reduce harmful metabolites, including bile acids, phenol, and ammonia, and influence dietary fat metabolism, which influences obesity. Changes in microbiota can occur rapidly after dietary changes. These effects can be both direct and indirect, that is, bacteria that can digest resistant starch generate energy, which provides them with a growth advantage in the gut (9). Changes in community composition can also occur from decreased pH, resulting from accumulation of SCFAs (108,109). Other by-products of resistant starch fermentation can be used by other classes of bacteria to enhance their abundance through metabolic cross-feeding (110).

Studies using rodent models revealed correlations between resistant starch diets and colonic pH, SCFA composition, and enzymatic activity associated with bacterial degradative pathways, and the abundance of several bacterial taxa (91). Studies have also been conducted on humans fed diets rich in resistant starches, which likewise revealed changes to the function or abundance of major groups of bacteria (111,112).

Because the vast majority of GI bacteria cannot presently be cultured *in vitro*, our understanding of the composition of GI microbiota has recently accelerated with the application of culture-independent bacterial community analysis (metagenomics) and next-generation sequencing technologies (113). Techniques associated with deep DNA sequencing include profiling the main taxa comprising complex bacterial communities by 16S rRNA gene sequencing or whole-genome sequencing that can also reveal functional changes within the community. DNA sequencing platforms associated with these approaches include pyrosequencing technology of Roche/454 systems and the more recent application of shorter-read Illumina sequencing (113). These systems have been used as part of large, comprehensive studies in both the United States (Human Microbiome Project) and Europe and China (Metagenomics of the Human Intestinal Tract Study) to characterize the microbiota from various locations, including the GI tract, from healthy adults (114–116). The Human Microbiome Project included both 16S rRNA gene phylotyping and whole-genome shotgun sequencing from multiple body sites over time (114), whereas the Metagenomics of the Human Intestinal Tract Study used shotgun metagenomic analysis of human fecal samples. The latter study revealed 3 distinct “enterotypes” characterized by their relative abundance of *Bacteroides*, *Prevotella*, and *Ruminococcus* genera in human populations (115). Although studies on this classification system are ongoing (117), diets that differ in fiber content appear to influence the composition of many of the major taxonomic units that produce SCFAs and offer beneficial effects to the host (118).

Martínez et al. (8) used pyrosequencing to characterize the impact of resistant starches on the composition of fecal microbiota in humans. Study participants consumed resistant starch representing either RSII (granular form of high-amylose corn starch) or RSIV, (chemically modified by phosphate cross-linking), which were compared with control starch in the form of crackers. Both forms of resistant starch increased representatives of the *Actinobacteria* and *Bacteroidetes* phyla and decreased *Firmicutes*. The 2 forms of resistant starch differed in their ability to change species. RSII increased the abundance of *R. bromii* and *E. rectale*, which was consistent with prior results from *in vitro* studies of starch fermentation in the large intestine (119,120). In contrast, RSIV was associated with increased *B. adolescentis* and *Parabacteroides distasonis* (8). Why the 2 different resistant starches change the composition of the microbiota remains unclear, because multiple genera are capable of degrading the starches (86,121). It was suggested that the differential ability for individual bacterial species to degrade

the starches may represent differences in substrate binding (8).

Individual study participants also appeared to have widely different responses to dietary starch (8,9). For example, in the Martínez et al. study (8) none of the taxa showed the same response in all 10 individuals, likely reflecting the known microbial variation throughout human populations (122,123). Strain differences in the ability to degrade the resistant starches (124) and host factor differences that distinguish the human participants also likely contribute to these changes. This finding is consistent with the observations that the products of GI fermentation (i.e., SCFAs) can vary greatly among individuals and their levels also correlate with diet (125,126).

Although deep-sequencing studies of the impact of resistant starches on colonic microbiota have only recently been initiated, they should help identify the mechanisms by which specific bacterial taxa interact with the different forms of starch. The interindividual variation in response to dietary resistant starch will also be an important area of investigation for the therapeutic and preventive use of resistant starch to improve human health (8).

Another topic of intense interest is the use of prebiotics to alter colonic microbiota to benefit the health of the host (127). Although typically associated with oligosaccharides, prebiotics can represent a variety of nondigestible carbohydrates, including resistant starch (128), that impart health benefits to the host through modulation of GI bacteria (129). Because of the interactions between GI microbiota and hosts, prebiotics, including resistant starches, have the potential to correct or prevent a variety of human diseases, including obesity, diabetes, inflammatory bowel diseases, and cancer (130,131).

Potential Impacts and Mechanisms of Action of Resistant Starch in Prevention of Colon Cancer

Consumption of diets with abundant fiber has long been believed to protect against colorectal cancer (132). More recently, resistant starch has received attention for potential prevention of colon cancer and inflammatory bowel diseases (104). Although studies of resistant starches and human colonic health have been limited, abstracts describing 2 recent human interventions were found. In 1, a 4-wk intervention with red meat (300 g/d) increased O⁶-methyl-2'-deoxyguanosine adducts and genes from the microRNA-17–92 cluster (overexpressed in colorectal cancer) in the colons of humans. However, these features were not elevated with a 4-wk intervention that included red meat plus butyrylated resistant starch (40 g/d) (133). These results suggested that resistant starch may protect the human colon against potentially damaging aspects of dietary red meat. In the second human trial, with hereditary nonpolyposis colorectal cancer gene carriers (patients with Lynch syndrome) at high risk for developing colon polyps and cancer, a diet containing 30 g/d maize starch (Novelose, Ingredion) was compared with placebo diet for 29 mo. No impact on polyp or colon cancer development was observed at a 4-y follow-up (134).

In contrast to the limited number of human interventions evaluating dietary resistant starch, several studies have been done on the impacts of resistant starch and colon cancer prevention in laboratory animals. Le Leu et al. (67,135,136) conducted extensive studies in rats treated with the colon carcinogen azoxymethane and/or fed diets high in protein to damage the colonic epithelium. Feeding high fiber or resistant starches increased fecal bulk, fecal pH, butyrate concentration, and epithelial apoptosis, and it decreased cell proliferation markers and colon carcinogenesis. When animals were fed high levels of protein, the addition of high-amylose resistant starch reduced protein fermentation products, which paralleled reduced colorectal carcinoma development. In contrast to these findings, studies with potato fiber or potato resistant starch in rats fed control protein or high-protein diets revealed no impact of dietary resistant starches on DNA damage in the colon (137). It is not clear whether the contrasting results are due to differences in experimental details, including the starches fed, or other factors. DNA damage was studied in rats fed diets with casein or soy protein and resistant starch (48% high-amylose maize starch), with attenuated DNA damage observed in rats fed high-protein diets and resistant starch (138). Butyrylated high-amylose corn starch was shown to be somewhat more effective than high-amylose corn starch in the inhibition of colon cancers in rats (139). Results from Zhao et al. (140), using a novel high-amylose starch complexed with steric acid (RSV) revealed a stunning reduction in mucin-depleted foci; however, subsequent studies found that mucin-depleted foci may not be a reliable marker for subsequent colon cancer (B Nelson, Y Zhao, N Cray, E Whitley, and DF Birt, unpublished results).

Numerous hypotheses have been proposed for the potential mechanism by which colon carcinogenesis may be altered by resistant starch. The most common hypotheses focus on alteration of the water-holding capacity of the fecal stream, modification of the microbiota, and increasing SCFA production. Although the SCFA hypothesis seems to have the most enthusiastic following, theories on the impacts of dietary resistant starch on the production of SCFAs and changing the microbiota are gaining momentum (141).

SCFAs (acetate, propionate, and butyrate) are increased in amount and concentration in many studies of resistant starch and colon health (104,132). Butyrate, an end-product of microbial fermentation of resistant starch and the primary energy source for colonocytes, is actively transported into cells by a Na^+ -dependent cotransporter (142). This cell membrane transporter serves as a tumor suppressor gene and is epigenetically silenced by hypermethylation in human aberrant crypt foci (a precancerous lesion) and colorectal cancer (143). Butyrate has been of particular interest because of the role this molecule plays in colonic epithelial metabolism and differentiation and its influence on signaling pathways that regulate mucosal physiology (141,144). In cell culture, butyrate has antitumorigenic properties, including reducing cell proliferation and inducing apoptosis of colorectal tumor cell lines (141). However, it is not clear whether the

concentrations of butyrate achieved in the colon of animals and humans fed resistant starches are optimal for the suppressive effects observed in cultured cells, because the concentrations of butyrate in animals fed control diets are higher than the concentrations of butyrate needed for suppression of colon proliferation (145).

Colon cancer appears to develop as the result of dysregulation of molecular pathways that control epithelial proliferation, maturation, and apoptosis, with perturbations of both genetic and epigenetic components. In regeneration of normal mucosa, stem cells deep within the crypt undergo mitosis, with subsequent maturation and differentiation of daughter cells to mature absorptive and secretory populations of the colonic mucosa and eventual loss of aged or damaged cells through apoptosis. Multistep accumulation of mutations of genes in critical control pathways, coupled with changes in epigenetic factors, is believed to allow survival of abnormal crypt epithelial cells with the potential to undergo malignant transformation.

The molecular mechanisms by which dietary resistant starches are believed to alter the development or progression of colon cancer are incompletely understood. Potential mechanisms of action of dietary resistant starch on gene expression and mutation, DNA methylation, histone modification, and remodeling of chromatin are being studied intensively. Recently, animal studies identified both alterations in the microbiota and induction of protection against unrepaired DNA damage by dietary high-amylose maize starch and butyrylated high-amylose, with increased expression of genes involved in repair of DNA (146), which, in rapidly dividing populations of the colonic mucosa, is expected to result in fewer mutations and reduced carcinogenesis. Butyrate has been also described to exert an influence on cell proliferation and differentiation through modulation of several signal transduction pathways (147). In some colon cancer cell lines, constitutive expression of the canonical *Wnt* pathway, an initiating event in most colorectal cancers, is upregulated by butyrate treatment, resulting in a strong apoptotic response (148). Butyrate has also been shown to influence gene expression in the colon by modulating RNA splicing (149).

The potential for modification of epigenetic mechanisms, by their nature potentially reversible, by metabolic products of resistant starch also holds promise for dietary prevention of colorectal cancer. Butyrate is well known as an inhibitor of histone deacetylase, an enzyme that modifies wrapping of strands of DNA around nuclear histone proteins and thereby regulates gene transcription. Inhibitors of histone deacetylation, such as butyrate, have the ability to modify expression of genes that control cell cycle and apoptosis and function to suppress the development of pre-neoplastic and neoplastic phenotypes *in vitro* (150). Furthermore, butyrate exerts protective effects against intestinal mucosal inflammation, a component of inflammation-mediated colorectal cancer, through apoptosis of T lymphocytes and inhibition of inducible nitric oxide synthase in colonic epithelium (151).

The potential contributions to colonic homeostasis by endogenous or microbial products of resistant starch metabolism

other than SCFAs are undetermined. Complex interactions between microbiota, dietary components, colonic epithelium, the immune system, and the nervous and endocrine systems are being dissected, and it is likely that mechanisms integrating these components will emerge.

Potential Impacts and Mechanisms of Action of Resistant Starch in Prevention or Therapy of Metabolic Diseases

Diabetes. Diabetes affects 8% of the United States population and 23% of the population >60 y of age, mostly as type 2 diabetes. Both type 1 and type 2 diabetes are characterized by hyperglycemia, subsequently resulting in systemic tissue toxicity. Some risk factors, including increased fasting and postprandial glucose response as well as decreased insulin sensitivity and obesity, are reversible through lifestyle modifications, which were found to be more effective than pharmacological interventions in delaying the onset of type 2 diabetes (152). One such lifestyle change is the replacement of ordinary starch in foods with resistant starch, owing to its low glycemic index. It has been reported in human studies that consuming foods, including corn porridges (153) and crackers (E Haugabrooks, Y-F Ai, J Jane, S Hendrich, unpublished results), with a high content of resistant starch resulted in lower postprandial glucose concentrations and concomitant insulin response compared with consuming foods containing ordinary starch (30,154). In addition, consuming less digestible starches may decrease glycemic response to a subsequent meal, the “second meal effect.” Ten healthy individuals who ate high-amylose starch at breakfast showed decreased blood glucose response to a lunch containing highly digestible carbohydrate, compared with eating high-amylopectin starch at breakfast (155). Consequently, replacing ordinary dietary starch with resistant starch contributes to diabetes management. Increasing consumption of resistant starch can also aid weight management, beneficially influence body composition, or both in part because food with resistant starch has lower energy concentration and has been shown in mice to reduce body fat, an important predictor of disease (156,157). Animal models of diabetes have also demonstrated a positive effect of dietary resistant starch, such as an improvement in glycemic control in the Goto-Kakizaki rat, a nonobese model of type 2 diabetes (65).

The potential for low glycemic index carbohydrates, such as resistant starch, to reduce diabetic complications may be related to protection of kidney function and lead to better maintenance of adequate nutritional status, particularly with respect to vitamin D. Numerous epidemiological and case-control studies have reported vitamin D insufficiency in type 1 and type 2 diabetes (158–163). However, it is unclear whether low vitamin D exposure contributed to the onset of diabetes or whether low vitamin D status was a consequence of diabetes. The major circulating form of vitamin D, 25-hydroxycholecalciferol (25D3), circulates bound to vitamin D-binding protein (DBP) until the 25D3-DBP complex is internalized through endocytosis and activated to 1,25-dihydroxycholecalciferol by the renal proximal tubule

cells or reabsorbed into the blood as 25D3 (164). In both type 1 and type 2 diabetic rats, excretion of 25D3 and DBP into the urine was markedly elevated as a result of pathologies associated with reduced expression of megalin and disabled-2, which partner together to facilitate the uptake of the 25D3-DBP complex by the kidneys (12,165). However, when cornstarch in the AIN-93G rodent diet was replaced with high-amylose maize, that is, ~37% resistant to digestion, urinary excretion of vitamin D metabolites and DBP was virtually prevented in diabetic rats (12). Of interest was the finding that feeding diabetic rats the resistant starch had only a slight effect on attenuating fasting blood glucose concentrations, yet the renal expression of megalin and disabled-2 were normal, as confirmed in a type 2 diabetes rat model (G Koh and M Rowling, unpublished results).

Obesity and body weight management. Overconsumption of energy is proposed to be responsible for the obesity epidemic, and, as a consequence, new strategies are required to reduce energy intake (166). One potential dietary strategy is to increase consumption of dietary fiber, which has been associated with increased satiety and lower BMI (167–169). Dietary fiber is a diverse group of carbohydrates, and large differences exist in their physical and chemical properties. Consequently, not all sources of fiber will have the same effect on satiety or body weight (167). Resistant starches are proposed to provide many of the benefits of dietary fiber; therefore, they may aid weight management, although it has yet to be adequately demonstrated.

Accumulating evidence from rodent studies suggests that replacing rapidly digestible starch with resistant starch reduces body weight. Aziz et al. (170) found that a diet high in resistant starch reduced body weight by 40% in diet-induced obese rats. However, the diet contained 23.4% resistant starch, an amount that may not be achievable in human diets. Another study fed rats a diet containing 4%, 8%, or 16% resistant starch and found that consuming a diet with >8% resistant starch reduced adiposity compared with 0%, and for every 4% increase in resistant starch, energy intake was reduced by 9.8 kJ/d (66). Long-term studies on the effect of increasing resistant starch consumption on body weight in humans are required.

Despite the lack of long-term studies, there are several reasons to believe that consuming resistant starch could aid weight management in humans. First, because of the lower calorie content, replacing rapidly digestible starch with resistant starch reduces the energy density of the diet (171,172). Several studies have found that reducing the energy density of the diet increases satiety and weight loss (173–176).

Second, incorporating resistant starch into a meal may augment feelings of satiety. Although fiber intake has been associated with increased satiety, the effect of resistant starch on satiety is less clear. In rodent models, adding resistant starch to the diet increased secretion of the putative satiety hormones GLP-1 and PYY (171,172), suggesting that it might augment satiety. The few studies that have been conducted in humans have provided mixed results. Willis et al.

(177) provided participants with low-fiber muffins or muffins supplemented with resistant starch for breakfast and found that consuming the muffins containing resistant starch promoted satiety and increased the duration of satiety. Bodinham et al. (178) fed males 48 g of resistant starch across 2 separate meals and found no effect on subjective appetite, although food intake was reduced by ~1300 kJ over 24 h. Conversely, a recent study that fed participants a breakfast meal containing 25 g of resistant starch had no effect on subjective appetite or food intake over the remainder of the day (179). This study also found that plasma concentrations of GLP-1 were lower after the resistant starch meal.

Third, resistant starch may influence body weight by increasing energy expenditure or fat oxidation. It has been proposed that replacing rapidly digestible starch with resistant starch may promote fat mobilization as the result of a reduction in insulin secretion (180). However, currently, little evidence supports this hypothesis and several studies have failed to show that resistant starch increases energy expenditure or fat oxidation (52,181,182).

Discussion

Digestion-resistant starches are categorized on the basis of their resistance to digestive enzymes and are the subject of investigation for inclusion in healthy foods. Environmental and genetic factors that affect starch resistance in crops are being identified, including using biotechnology to control starch digestibility. Although both in vivo digestion and in vitro digestion of starches have received considerable attention, there is a need for improved, validated in vitro methods that reflect in vivo digestion under the myriad conditions the starch may encounter in animal and human digestion. Further, although several challenges have been identified in attempts to incorporate resistant starch into human diets, much work must still be done to effectively overcome the identified barriers.

The complexity of resistant starch effects on gut microbiota and microbial effects on resistant starch metabolism has been the focus of much work. With the application of new genomic techniques to gut microbiota, the pace of progress is anticipated to accelerate. In addition, to best interpret gut microbiota data, we need advances to expand the capabilities of resistant starch analytical methods, which still depend greatly on methods developed earlier for dietary fiber.

Finally, studies of resistant starch on disease processes, including colon cancer, diabetes, and obesity, show promise, and intriguing hypotheses have been developed. However, there is a need for considerable research to identify the potential effectiveness of digestion-resistant starches in the prevention and control of human diseases and to identify mechanisms underpinning their actions. In particular, considering the tremendous diversity of digestion-resistant starches in plants, very few of these starches have been studied for their effects on animals or humans. Future integrative research that addresses all of these fronts will help expand the potential uses for digestion-resistant starches in health promotion.

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Literature Cited

- Englyst HN, Kingman SM, Cummings JH. Classification and measurement of nutritionally important starch fractions. *Eur J Clin Nutr.* 1992;46(Supp 2):S33–50.
- Sajilata MG, Singhal RS, Kulkarni PR. Resistant starch—A review. *Compr Rev Food Sci Food Saf.* 2006;5:1–17.
- Nugent AP. Health properties of resistant starch. *Nutr Bull.* 2005;30:27–54.
- Thompson DB. Strategies for the manufacture of resistant starch. *Trends Food Sci Technol.* 2000;11:245–53.
- Fuentes-Zaragoza E, Sanchez-Zapata E, Sendra E, Sayas E, Navarro C, Fernandez-Lopez J, Perez-Alvarez JA. Resistant starch as prebiotic: A review. *Starch-Stärke.* 2011;63:406–15.
- Brouns F, Kettlitz B, Arrigoni E. Resistant starch and "the butyrate revolution." *Trends Food Sci Technol.* 2002;13:251–61.
- Murphy MM, Douglass JS, Birkett A. Resistant starch intakes in the United States. *J Am Diet Assoc.* 2008;108:67–78.
- Martínez I, Kim J, Duffy P, Schlegel V, Walter J. Resistant starches types 2 and 4 have differential effects on the composition of the fecal microbiota in human subjects. *PLoS ONE.* 2010;5:e15046.
- Walker AW, Ince J, Duncan S, Webster L, Holtrop G, Ze X, Brown D, Stares M, Scott P, Bergerat A, et al. Dominant and diet-responsive groups of bacteria within the human colonic microbiota. *ISME J.* 2011;5:220–30.
- Wu GD, Chen YY, Hoffmann C, Bittinger K, Chen Y, Keilbaugh SA, Bewtra M, Knights D, Walters WA, Knight R, et al. Linking long-term dietary patterns with gut microbial enterotypes. *Science.* 2011;334:105–8.
- Birt DF, Phillips GJ. Diet, genes and microbes; complexities of colon cancer prevention. *Toxicol Pathol.* 2013; in press.
- Smazal AL, Borcharding NC, Anderreg AS, Whitley EM, Schalinske KL, Rowling MJ. Dietary resistant starch prevents urinary excretion of 25-hydroxycholecalciferol and vitamin D-binding protein in type 1 diabetic rats. *J Nutr.* 2013;143:1123–8.
- Englyst HN, Cummings JH. Digestion of the polysaccharides of some cereal foods in the human small-intestine. *Am J Clin Nutr.* 1985;42:778–87.
- O'Dea K, Nestel PJ, Antonoff L. Physical factors influencing postprandial glucose and insulin responses to starch. *Am J Clin Nutr.* 1980;33:760–5.
- Jenkins DJA, Wesson V, Wolever TMS, Jenkins AL, Kalmusky J, Guidici S, Csuma A, Josse RG, Wong GS. Wholemeal versus wholegrain breads - proportion of whole or cracked grain and the glycemic response. *BMJ.* 1988;297:958–60.
- Granfeldt Y, Bjorck I, Hagander B. On the importance of processing conditions, product thickness and egg addition for the glycemic and hormonal responses to pasta - a comparison with bread made from pasta ingredients. *Eur J Clin Nutr.* 1991;45:489–99.
- Jane JL, Ao Z, Duvick SA, Wiklund M, Yoo S-H, Wong K-S, Gardner C. Structures of amylopectin and starch granules: How are they synthesized? *J Appl Glycosci.* 2003;50:167–72.
- Regina A, Bird A, Topping D, Bowden S, Freeman J, Barsby T, Kosar-Hashemi B, Li Z, Rahman S, Morell M. High-amylose wheat generated by RNA interference improves indices of large-bowel health in rats. *Proc Natl Acad Sci USA.* 2006;103:3546–51.
- Li L, Jiang HX, Campbell M, Blanco M, Jane JL. Characterization of maize amylose-extender (ae) mutant starches. Part I: Relationship between resistant starch contents and molecular structures. *Carbohydr Polym.* 2008;74:396–404.
- Jiang H, Campbell M, Blanco M, Jane J-L. Characterization of maize amylose-extender (ae) mutant starches: Part II. Structures and properties of starch residues remaining after enzymatic hydrolysis at boiling-water temperature. *Carbohydr Polym.* 2010;80:1–12.
- Jane J, Robyt JF. Structure studies of amylose- α complexes and retrograded amylose by action of α -amylases, and a new method for preparing amyloextrins. *Carbohydr Res.* 1984;132:105–18.

22. Witt T, Gidley MJ, Gilbert RG. Starch digestion mechanistic information from the time evolution of molecular size distributions. *J Agric Food Chem.* 2010;58:8444–52.
23. Sievert D, Pomeranz Y. Enzyme-resistant starch. 2. Differential scanning calorimetry studies on heat-treated starches and enzyme-resistant starch residues. *Cereal Chem.* 1990;67:217–21.
24. Woo KS, Seib PA. Cross-linked resistant starch: preparation and properties. *Cereal Chem.* 2002;79:819–25.
25. Han J-A, BeMiller JN. Preparation and physical characteristics of slowly digesting modified food starches. *Carbohydr Polym.* 2007;67:366–74.
26. He J, Liu J, Zhang G. Slowly digestible waxy maize starch prepared by octenyl succinic anhydride esterification and heat-moisture treatment: glycemic response and mechanism. *Biomacromolecules.* 2008;9:175–84.
27. Zhang B, Huang Q, Luo F-X, Fu X, Jiang H, Jane J-I. Effects of octenylsuccinylation on the structure and properties of high-amylose maize starch. *Carbohydr Polym.* 2011;84:1276–81.
28. Annison G, Illman RJ, Topping DL. Acetylated, propionylated or butyrylated starches raise large bowel short-chain fatty acids preferentially when fed to rats. *J Nutr.* 2003;133:3523–8.
29. Ai Y, Hasjim J, Jane J-I. Effects of lipids on enzymatic hydrolysis and physical properties of starch. *Carbohydr Polym.* 2013;92:120–7.
30. Hasjim J, Lee S-O, Hendrich S, Setiawan S, Ai Y, Jane J-I. Characterization of a novel resistant-starch and its effects on postprandial plasma-glucose and insulin responses. *Cereal Chem.* 2010;87:257–62.
31. Seneviratne HD, Biliaderis CG. Action of α -amylases on amylose-lipid complex superstructures. *J Cereal Sci.* 1991;13:129–43.
32. Krieger KM, Pollak LM, Brumm TJ, White PJ. Effects of pollination method and growing location on starch thermal properties of corn hybrids. *Cereal Chem.* 1998;75:656–9.
33. Pollak LM, Scott MP, Duvick SA. Resistant starch and starch thermal characteristics in exotic corn lines grown in temperate and tropical environments. *Cereal Chem.* 2011;88:435–40.
34. Keeling PL, Banisadr B, Barone L, Wasserman BP, Singletary GW. Effect of temperature on enzymes in the pathway of starch biosynthesis in developing wheat and maize grain. *Aust J Plant Physiol.* 1994;21:807–27.
35. Rohlffing KA, Pollak LM, White PJ. Exotic corn lines with increased resistant starch and impact on starch thermal characteristics. *Cereal Chem.* 2010;87:190–3.
36. Stinard PS, Robertson DS, Schnable PS. Genetic isolation, cloning, and analysis of a mutator-induced, dominant antimorph of the maize amylose extender1 locus. *Plant Cell.* 1993;5:1555–66.
37. Garwood DL, Shannon JC, Creech RG. Starches of endosperms possessing different alleles at the amylose-extender locus in *Zea mays* L. *Cereal Chem.* 1973;53(3):355–364. Available from: http://www.aaccnet.org/publications/cc/backissues/1976/Documents/Chem53_355.pdf.
38. Campbell MR, Jane J-I, Pollak L, Blanco M, O'Brien A. Registration of maize germplasm line GEMS-0067. *J Plant Regist.* 2007;1:60–1.
39. Chen T, Ning L, Liu X, Cui D, Zhang H, Li D, Zhao L, Chen H. Development of functional molecular markers of SbeI and SbeIIb for the high amylose maize germplasm line GEMS-0067. *Crop Sci.* 2013;53:482–90.
40. Morell MK, Kosar-Hashemi B, Cmiel M, Samuel MS, Chandler P, Rahman S, Buleon A, Batey IL, Li ZY. Barley sex6 mutants lack starch synthase IIa activity and contain a starch with novel properties. *Plant J.* 2003;34:173–185.
41. Bird AR, Vuaran MS, King RA, Noakes M, Keogh J, Morell MK, Topping DL. Wholegrain foods made from a novel high-amylose barley variety (Himalaya 292) improve indices of bowel health in human subjects. *Br J Nutr.* 2008;99:1032–40.
42. Jobling SA, Schwall GP, Westcott RJ, Sidebottom CM, Debet M, Gidley MJ, Jeffcoat R, Safford R. A minor form of starch branching enzyme in potato (*Solanum tuberosum* L.) tubers has a major effect on starch structure: cloning and characterisation of multiple forms of SBE A. *Plant J.* 1999;18:163–71.
43. Schwall GP, Safford R, Westcott RJ, Jeffcoat R, Tayal A, Shi Y-C, Gidley MJ, Jobling SA. Production of very-high-amylose potato starch by inhibition of SBE A and B. *Nat Biotechnol.* 2000;18:551–4.
44. Blennow A, Wischmann B, Houborg K, Ahmt T, Jørgensen K, Engelsen SB, Bandsholm O, Poulsen P. Structure function relationships of transgenic starches with engineered phosphate substitution and starch branching. *Int J Biol Macromol.* 2005;36:159–68.
45. Sestili F, Janni M, Doherty A, Botticella E, D'Ovidio R, Masci S, Jones H, Lafiandra D. Increasing the amylose content of durum wheat through silencing of the SBEIIa genes. *BMC Plant Biol.* 2010;10:144.
46. Wei C, Xu B, Qin F, Yu H, Chen C, Meng X, Zhu L, Wang Y, Gu M, Liu Q. C-type starch from high-amylose rice resistant starch granules modified by antisense RNA inhibition of starch branching enzyme. *J Agric Food Chem.* 2010;58:7383–8.
47. Carciofi M, Blennow A, Jensen S, Shaik S, Henriksen A, Buleon A, Holm P, Hebelstrup K. Concerted suppression of all starch branching enzyme genes in barley produces amylose-only starch granules. *BMC Plant Biol.* 2012;12:223.
48. The definition of dietary fiber. *Cereal Foods World.* 2001;46:112–26.
49. AOAC. Official methods of analysis of AOAC International. [cited 2013 Jan 4]. Available from: <http://www.eoma.aoc.org/>.
50. Englyst HN, Quigley ME, Hudson GJ. Determination of dietary fibre as non-starch polysaccharides with gas-liquid chromatographic, high-performance liquid chromatographic or spectrophotometric measurement of constituent sugars. *Analyst (Lond).* 1994;119:1497–509.
51. Vonk RJ, Hagedoorn RE, Elzinga H, Tabak S, Yang Y, Stellaard F. Digestion of so-called resistant starch sources in the human small intestine. *Am J Clin Nutr.* 2000;72:432–8.
52. Tagliabue A, Raben A, Heijnen ML, Deurenberg P, Pasquali E, Astrup A. The effect of raw potato starch on energy-expenditure and substrate oxidation. *Am J Clin Nutr.* 1995;61:1070–5.
53. Faisant N, Buleon A, Colonna P, Molis C, Lartigue S, Galmiche JB, Champ M. Digestion of raw banana starch in the small-intestine of healthy humans - structural features of resistant starch. *Br J Nutr.* 1995;73:111–23.
54. Englyst HN, Cummings JH. Digestion of polysaccharides of potato in the small-intestine of man. *Am J Clin Nutr.* 1987;45:423–31.
55. Muir JG, Odea K. Validation of an in vitro assay for predicting the amount of starch that escapes digestion in the small-intestine of humans. *Am J Clin Nutr.* 1993;57:540–6.
56. Silvester KR, Englyst HN, Cummings JH. Ileal recovery of starch from whole diets containing resistant starch measured in-vitro and fermentation of ileal effluent. *Am J Clin Nutr.* 1995;62:403–11.
57. Mathers JC, Smith H, Carter S. Dose-response effects of raw potato starch on small-intestinal escape, large-bowel fermentation and gut transit time in the rat. *Br J Nutr.* 1997;78:1015–29.
58. Giuberti G, Gallo A, Masoero F. Plasma glucose response and glycemic indices in pigs fed diets differing in in vitro hydrolysis indices. *Animal.* 2012;6:1068–76.
59. Jarret G, Cerisuelo A, Peu P, Martinez J, Dourmad J-Y. Impact of pig diets with different fibre contents on the composition of excreta and their gaseous emissions and anaerobic digestion. *Agric Ecosyst Environ.* 2012;160:51–8.
60. Gidenne T, Perez JM. Effect of dietary starch origin on digestion in the rabbit. 1. Digestibility measurements from weaning to slaughter. *Anim Feed Sci Technol.* 1993;42:237–47.
61. Hägele EO, Schaich E, Rauscher E, Lehmann P, Burk H, Wahlefeld AW. Mechanism of action of human pancreatic and salivary alpha-amylase on alpha-4-nitrophenyl maltoheptaoside substrate. *Clin Chem.* 1982;28:2201–5.
62. Abdullah M, French D, Robyt JF. Multiple attack by alpha-amylases. *Arch Biochem Biophys.* 1966;114:595–8.
63. Pasero L, Mazzeippierron Y, Abadie B, Chicheportiche Y, Marchismouren G. Complete amino-acid-sequence and location of the 5 disulfide bridges in porcine pancreatic alpha-amylase. *Biochim Biophys Acta.* 1986;869:147–57.
64. Sugimoto Y, Fujita S, Takaya T, Fuwa H. In vivo digestion of starch granules by rats. 3. In vivo digestion of banana starch granules. *Stärke.* 1980;32:290–4.
65. Shen L, Keenan MJ, Reggio A, Williams C, Martin RJ. Dietary-resistant starch improves maternal glycemic control in Goto-Kakizaki rat. *Mol Nutr Food Res.* 2011;55:1499–508.

66. Belobrajdic DP, King RA, Christophersen CT, Bird AR. Dietary resistant starch dose-dependently reduces adiposity in obesity-prone and obesity-resistant male rats. *Nutr Metab (Lond)*. 2012;9(1):93.
67. Le Leu RK, Brown IL, Hu Y, Morita T, Esterman A, Young GP. Effect of dietary resistant starch and protein on colonic fermentation and intestinal tumorigenesis in rats. *Carcinogenesis*. 2007;28:240–5.
68. Anderson TJ, Ai Y, Jones RW, Houk RS, Jane J-I, Zhao Y, Birt DF, McClelland JF. Analysis of resistant starches in rat cecal contents using Fourier transform infrared photoacoustic spectroscopy. *J Agric Food Chem*. 2013;61:1818–22.
69. Goñi I, Garcia-Diz L, Mañas E, Saura-Calixto F. Analysis of resistant starch: a method for foods and food products. *Food Chem*. 1996;56:445–9.
70. Jenkins DJA, Wolever TMS, Taylor RH, Barker H, Fielden H, Baldwin JM, Bowling AC, Newman HC, Jenkins AL, Goff DV. Glycemic index of foods: a physiological basis for carbohydrate exchange. *Am J Clin Nutr*. 1981;34:362–6.
71. Hallström E, Sestili F, Lafiandra D, Bjorck I, Ostman E. A novel wheat variety with elevated content of amylose increases resistant starch formation and may beneficially influence glycaemia in healthy subjects. *Food Nutr Res*. 2011;55: 10.3402/fnr.v55i0.7074.
72. Sharma A, Yadav BS, Ritika. Resistant starch: physiological roles and food applications. *Food Rev Int*. 2008;24:193–234.
73. Faraj A, Vasanthan T, Hoover R. The effect of extrusion cooking on resistant starch formation in waxy and regular barley flours. *Food Res Int*. 2004;37:517–25.
74. Wang JS, Rosell CM, de Barber CB. Effect of the addition of different fibres on wheat dough performance and bread quality. *Food Chem*. 2002;79:221–6.
75. Van Hung P, Yamamori M, Morita N. Formation of enzyme-resistant starch in bread as affected by high-amylose wheat flour substitutions. *Cereal Chem*. 2005;82:690–4.
76. Korus J, Witzczak M, Ziobro R, Juszczak L. The impact of resistant starch on characteristics of gluten-free dough and bread. *Food Hydrocoll*. 2009;23:988–95.
77. Riva M, Fessas D, Schiraldi A. Starch retrogradation in cooked pasta and rice. *Cereal Chem*. 2000;77:433–8.
78. Sanz T, Salvador A, Baixauli R, Fiszman SM. Evaluation of four types of resistant starch in muffins. II. Effects in texture, colour and consumer response. *Eur Food Res Technol*. 2009;229:197–204.
79. Rohlfing KA, Paez A, Kim HJ, White PJ. Effects of resistant starch and fiber from high-amylose non-floury corn on tortilla texture. *Cereal Chem*. 2010;87:581–5.
80. Baixauli R, Sanz T, Salvador A, Fiszman SM. Muffins with resistant starch: baking performance in relation to the rheological properties of the batter. *J Cereal Sci*. 2008;47:502–9.
81. Baixauli R, Salvador A, Fiszman SM. Textural and colour changes during storage and sensory shelf life of muffins containing resistant starch. *Eur Food Res Technol*. 2008;226:523–30.
82. Topping DL, Clifton PM. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiol Rev*. 2001;81:1031–64.
83. Erra-Pujada M, Debeire P, Duchiron F, O'Donohue MJ. The type II pullulanase of *Thermococcus hydrothermalis*: molecular characterization of the gene and expression of the catalytic domain. *J Bacteriol*. 1999;181:3284–7.
84. Macfarlane GT, Englyst HN. Starch utilization by the human large intestinal microflora. *J Appl Bacteriol*. 1986;60:195–201.
85. Wang X, Conway PL, Brown IL, Evans AJ. In vitro utilization of amylopectin and high-amylose maize (amylomaize) starch granules by human colonic bacteria. *Appl Environ Microbiol*. 1999;65:4848–54.
86. Flint HJ, Bayer EA, Rincon MT, Lamed R, White BA. Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. *Nat Rev Microbiol*. 2008;6:121–31.
87. Reeves AR, Wang GR, Salyers AA. Characterization of four outer membrane proteins that play a role in utilization of starch by *Bacteroides thetaiotaomicron*. *J Bacteriol*. 1997;179:643–9.
88. Li L. Assessing prebiotic effects of resistant starch on modulating gut microbiota with an in vivo animal model and in vitro semi-continuous fermentation model. Ames, IA: Iowa State University; 2010.
89. Barcenilla A, Pryde SE, Martin JC, Duncan SH, Stewart CS, Henderson C, Flint HJ. Phylogenetic relationships of butyrate-producing bacteria from the human gut. *Appl Environ Microbiol*. 2000;66:1654–61.
90. Pryde SE, Duncan SH, Hold GL, Stewart CS, Flint HJ. The microbiology of butyrate formation in the human colon. *FEMS Microbiol Lett*. 2002;217:133–9.
91. Louis P, Scott KP, Duncan SH, Flint HJ. Understanding the effects of diet on bacterial metabolism in the large intestine. *J Appl Microbiol*. 2007;102:1197–208.
92. Zhu Y, Liu XG, Yang ST. Construction and characterization of pta gene-deleted mutant of *Clostridium tyrobutyricum* for enhanced butyric acid fermentation. *Biotechnol Bioeng*. 2005;90:154–66.
93. Duncan SH, Hold GL, Barcenilla A, Stewart CS, Flint HJ. *Roseburia intestinalis* sp nov., a novel saccharolytic, butyrate-producing bacterium from human faeces. *Int J Syst Evol Microbiol*. 2002;52:1615–20.
94. Belenguer A, Duncan SH, Calder AG, Holtrop G, Louis P, Lobley GE, Flint HJ. Two routes of metabolic cross-feeding between *Bifidobacterium adolescentis* and butyrate-producing anaerobes from the human gut. *Appl Environ Microbiol*. 2006;72:3593–9.
95. Louis P, Duncan SH, McCrae SI, Millar J, Jackson MS, Flint HJ. Restricted distribution of the butyrate kinase pathway among butyrate-producing bacteria from the human colon. *J Bacteriol*. 2004;186:2099–106.
96. Louis P, Young P, Holtrop G, Flint HJ. Diversity of human colonic butyrate-producing bacteria revealed by analysis of the butyryl-CoA:acetate CoA-transferase gene. *Environ Microbiol*. 2010;12:304–14.
97. Gill SR, Pop M, DeBoy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, Gordon JI, Relman DA, Fraser-Liggett CM, Nelson KE. Metagenomic analysis of the human distal gut microbiome. *Science*. 2006;312:1355–9.
98. Pitt P, Debruijn KM, Beeching MF, Goldberg E, Blendis LM. Studies on breath methane the effect of ethnic origins and lactulose. *Gut*. 1980;21:951–4.
99. Abell GCJ, Conlon MA, McOrist AL. Methanogenic archaea in adult human faecal samples are inversely related to butyrate concentration. *Microb Ecol Health Dis*. 2006;18:154–60.
100. Weaver GA, Krause JA, Miller TL, Wolin MJ. Cornstarch fermentation by the colonic microbial community yields more butyrate than does cabbage fiber fermentation; cornstarch fermentation rates correlate negatively with methanogenesis. *Am J Clin Nutr*. 1992;55:70–7.
101. Sekirov I, Russell SL, Antunes CM, Finlay BB. Gut microbiota in health and disease. *Physiol Rev*. 2010;90:859–904.
102. Gonzalez A, Stombaugh J, Lozupone C, Turnbaugh PJ, Gordon JI, Knight R. The mind-body-microbial continuum. *Dialogues Clin Neurosci*. 2011;13:55–62.
103. Bounnik Y, Raskine L, Simoneau G, Vicaud E, Neut C, Flourié B, Brouns F, Bornet FR. The capacity of nondigestible carbohydrates to stimulate fecal bifidobacteria in healthy humans: a double-blind, randomized, placebo-controlled, parallel-group, dose-response relation study. *Am J Clin Nutr*. 2004;80:1658–64.
104. Higgins JA. Resistant starch: a promising dietary agent for the prevention/treatment of inflammatory bowel disease and bowel cancer. *Curr Opin Gastroenterol*. 2013;29:190–4.
105. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. *Nature*. 2006;444:1022–3.
106. Macfarlane S, Macfarlane GT. Regulation of short-chain fatty acid production. *Proc Nutr Soc*. 2003;62:67–72.
107. Cummings JH, Macfarlane GT. The control and consequences of bacterial fermentation in the human colon. *J Appl Bacteriol*. 1991;70:443–59.
108. El Oufir L, Flourié B, Bruley des Varannes S, Barry JL, Cloarec D, Bornet F, Galmiche JP. Relations between transit time, fermentation products, and hydrogen consuming flora in healthy humans. *Gut*. 1996;38:870–7.
109. Lewis SJ, Heaton KW. Increasing butyrate concentration in the distal colon by accelerating intestinal transit. *Gut*. 1997;41:245–51.

110. Flint HJ, Duncan SH, Scott KP, Louis P. Interactions and competition within the microbial community of the human colon: links between diet and health. *Environ Microbiol.* 2007;9:1101–11.
111. Phillips J, Muir JG, Birkett A, Lu ZX, Jones GP, O'Dea K, Young GP. Effect of resistant starch on fecal bulk and fermentation-dependent events in humans. *Am J Clin Nutr.* 1995;62:121–30.
112. Tomlin J, Read NW. The effect of resistant starch on colon function in humans. *Br J Nutr.* 1990;64:589–95.
113. Weinstock GM. Genomic approaches to studying the human microbiota. *Nature.* 2012;489:250–6.
114. The Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature.* 2012;486:207–14.
115. Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, Fernandes GR, Tap J, Bruls T, Batto JM, et al. Enterotypes of the human gut microbiome. *Nature.* 2011;473:174–80.
116. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature.* 2010;464:59–65.
117. Jeffery IB, Claesson MJ, O'Toole PW, Shanahan F. Categorization of the gut microbiota: enterotypes or gradients? *Nat Rev Microbiol.* 2012;10:591–2.
118. Maslowski KM, Mackay CR. Diet, gut microbiota and immune responses. *Nat Immunol.* 2011;12:5–9.
119. Kovatcheva-Datchary P, Egert M, Maathuis A, Rajilic-Stojanovic M, de Graaf AA, Smidt H, de Vos WM, Venema K. Linking phylogenetic identities of bacteria to starch fermentation in an in vitro model of the large intestine by RNA-based stable isotope probing. *Environ Microbiol.* 2009;11:914–26.
120. Leitch EC, Walker AW, Duncan SH, Holtrop G, Flint HJ. Selective colonization of insoluble substrates by human faecal bacteria. *Environ Microbiol.* 2007;9:667–79.
121. Flint HJ, Scott KP, Duncan SH, Louis P, Forano E. Microbial degradation of complex carbohydrates in the gut. *Gut Microbes.* 2012;3:289–306.
122. Tap J, Mondot S, Levenez F, Pelletier E, Caron C, Furet JB, Ugarte E, Muñoz-Tamayo R, Paslier DL, Nalin R, et al. Towards the human intestinal microbiota phylogenetic core. *Environ Microbiol.* 2009;11:2574–84.
123. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, et al. A core gut microbiome in obese and lean twins. *Nature.* 2009;457:480–4.
124. Ramsay AG, Scott KP, Martin JC, Rincon MT, Flint HJ. Cell-associated alpha-amylases of butyrate-producing Firmicute bacteria from the human colon. *Microbiology.* 2006;152:3281–90.
125. McOrist AL, Miller R, Bird A, Keogh J, Noakes M, Topping D, Conlon M. Fecal butyrate levels vary widely among individuals but are usually increased by a diet high in resistant starch. *J Nutr.* 2011;141:883–9.
126. Cummings JH, Beatty E, Kingman S, Bingham S, Englyst H. Digestion and physiological properties of resistant starch in the human large bowel. *Br J Nutr.* 1996;75:733–47.
127. Delzenne NM, Neyrinck AM, Cani PD. Gut microbiota and metabolic disorders: how prebiotic can work? *Br J Nutr.* 2013;109:S81–5.
128. Slavin J. Fiber and prebiotics: mechanisms and health benefits. *Nutrients.* 2013;5:1417–35.
129. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota—introducing the concept of prebiotics. *J Nutr.* 1995;125:1401–12.
130. DuPont AW, DuPont HL. The intestinal microbiota and chronic disorders of the gut. *Nat Rev Gastroenterol Hepatol.* 2011;8:523–31.
131. Holmes E, Kinross J, Gibson GR, Burcelin R, Jia W, Pettersson S, Nicholson JK. Therapeutic modulation of microbiota-host metabolic interactions. *Sci Transl Med.* 2012;6:137rv6.
132. Zhang G, Hamaker BR. Cereal carbohydrates and colon health. *Cereal Chem.* 2010;87:331–41.
133. Le Leu R, Conlon M, Winter J, Humphreys K, Michael M, Hu Y, Bird A, Topping D, Young G. Effect of high red meat intake and resistant starch in humans on risk factors for colorectal cancer. *J Gastroenterol Hepatol.* 2012;27:24–5.
134. Burn J, Bishop DT, Mecklin J, Macrae F, Moeslein G, Olschwang S, Bisgaard M, Ramesar R, Elliott F, Mathers J. Results of the CAPP-2-trial (aspirin and resistant starch) in HNPCC gene carriers. *EJC Suppl.* 2008;6:25.
135. Le Leu RK, Hu Y, Young GP. Effects of resistant starch and nonstarch polysaccharides on colonic luminal environment and genotoxin-induced apoptosis in the rat. *Carcinogenesis.* 2002;23:713–9.
136. Le Leu RKB, Brown IL, Hu Y, Esterman A, Young GP. Suppression of azoxymethane-induced colon cancer development in rats by dietary resistant starch. *Cancer Biol Ther.* 2007;6:1621–6.
137. Paturi G, Nyanhanda T, Butts CA, Herath TD, Monroe JA, Ansell J. Effects of potato fiber and potato-resistant starch on biomarkers of colonic health in rats fed diets containing red meat. *J Food Sci.* 2012;77:H216–23.
138. Toden S, Bird AR, Topping DL, Conlon MA. Differential effects of dietary whey, casein and soya on colonic DNA damage and large bowel SCFA in rats fed diets low and high in resistant starch. *Br J Nutr.* 2007;97:535–43.
139. Clarke JM, Topping DL, Bird AR, Young GP, Cobiac L. Effects of high-amylose maize starch and butyrylated high-amylose maize starch on azoxymethane-induced intestinal cancer in rats. *Carcinogenesis.* 2008;29:2190–4.
140. Zhao Y, Hasjim J, Li L, Jane J-L, Hendrich S, Birt DF. Inhibition of azoxymethane-induced preneoplastic lesions in the rat colon by a cooked stearic acid complexed high-amylose cornstarch. *J Agric Food Chem.* 2011;59:9700–8.
141. Fung KY, Cosgrove L, Lockett T, Head R, Topping DL. A review of the potential mechanisms for the lowering of colorectal oncogenesis by butyrate. *Br J Nutr.* 2012;108:820–31.
142. Gupta N, Martin PM, Prasad PD, Ganapathy V. SLC5A8 (SMCT1)-mediated transport of butyrate forms the basis for the tumor suppressive function of the transporter. *Life Sci.* 2006;78:2419–25.
143. Li H, Myeroff L, Smiraglia D, Romero MF, Pretlow TP, Kasturi L, Lutterbaugh J, Rerko RM, Casey G, Issa JP, et al. SLC5A8, a sodium transporter, is a tumor suppressor gene silenced by methylation in human colon aberrant crypt foci and cancers. *Proc Natl Acad Sci USA.* 2003;100:8412–7.
144. Nepelska M, Cultrone A, Beguet-Crespel F, Le Roux K, Dore J, Arulampalam V, Blottiere HM. Butyrate produced by commensal bacteria potentiates phorbol esters induced AP-1 response in human intestinal epithelial cells. *PLoS ONE.* 2012;7:e52869.
145. Macfarlane GT, Macfarlane S. Bacteria, colonic fermentation, and gastrointestinal health. *J AOAC Int.* 2012;95:50–60.
146. Conlon MA, Kerr CA, McSweeney CS, Dunne RA, Shaw JM, Kang S, Bird AR, Morell MK, Lockett TJ, Molloy PL, et al. Resistant starches protect against colonic DNA damage and alter microbiota and gene expression in rats fed a Western diet. *J Nutr.* 2012;142:832–40.
147. Bordonaro M, Mariadason JM, Aslam F, Heerdt BG, Augenlicht LH. Butyrate-induced apoptotic cascade in colonic carcinoma cells: modulation of the beta-catenin-Tcf pathway and concordance with effects of sulindac and trichostatin A but not curcumin. *Cell Growth Differ.* 1999;10:713–20.
148. Lazarova DL, Bordonaro M, Carbone R, Sartorelli AC. Linear relationship between Wnt activity levels and apoptosis in colorectal carcinoma cells exposed to butyrate. *Int J Cancer.* 2004;110:523–31.
149. Bordonaro M. Crosstalk between Wnt signaling and RNA processing in colorectal cancer. *J Cancer.* 2013;4:96–103.
150. Le Leu RK, Hu Y, Brown IL, Young GP. Effect of high amylose maize starches on colonic fermentation and apoptotic response to DNA-damage in the colon of rats. *Nutr Metab (Lond).* 2009;6:11.
151. Zimmerman MA, Singh N, Martin PM, Thangaraju M, Ganapathy V, Waller JL, Shi H, Robertson KD, Munn DH, Liu K. Butyrate suppresses colonic inflammation through HDAC1-dependent Fas upregulation and Fas-mediated apoptosis of T cells. *Am J Physiol Gastrointest Liver Physiol.* 2012;302:G1405–15.
152. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM. Diabetes Prevention Program Res G.

- Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med.* 2002;346:393–403.
153. Alexander D. Postprandial effects of resistant starch corn porridges on blood glucose and satiety responses in non-overweight and overweight adults. Ames, IA: Iowa State University; 2012.
 154. Kendall CWC, Esfahani A, Sanders LM, Potter SM, Vidgen E. The effect of a pre-load meal containing resistant starch on spontaneous food intake and glucose and insulin responses. *J Food Technol.* 2010;8:67–73.
 155. Brighenti F, Benini L, Del Rio D, Casiraghi C, Pellegrini N, Scazzina F, Jenkins DJA, Vantini I. Colonic fermentation of indigestible carbohydrates contributes to the second-meal effect. *Am J Clin Nutr.* 2006;83:817–22.
 156. So PW, Yu WS, Kuo YT, Wasserfall C, Goldstone AP, Bell JD, Frost G. Impact of resistant starch on body fat patterning and central appetite regulation. *PLoS ONE.* 2007;2:e1309.
 157. Behall KM, Howe JC. Resistant starch as energy. *J Am Coll Nutr.* 1996;15:248–54.
 158. Mutlu A, Mutlu GY, Ozsu E, Cizmecioglu FM, Hatun S. Vitamin D deficiency in children and adolescents with type 1 diabetes. *J Clin Res Pediatr Endocrinol.* 2011;3:179–83.
 159. McGill AT, Stewart JM, Lithander FE, Strik CM, Poppitt SD. Relationships of low serum vitamin D3 with anthropometry and markers of the metabolic syndrome and diabetes in overweight and obesity. *Nutr J.* 2008;7:4.
 160. Hurskainen AR, Virtanen JK, Tuomainen TP, Nurmi T, Voutilainen S. Association of serum 25-hydroxyvitamin D with type 2 diabetes and markers of insulin resistance in a general older population in Finland. *Diabetes Metab Res Rev.* 2012;28:418–23.
 161. Husemoen LL, Skaaby T, Thuesen BH, Jorgensen T, Fenger RV, Linneberg A. Serum 25(OH)D and incident type 2 diabetes: a cohort study. *Eur J Clin Nutr.* 2012;66:1309–14.
 162. Alam U, Najam O, Al-Himidani S, Benoliel S, Jinadev P, Berry JL, Kew M, Asghar O, Petropoulos IN, Malik RA. Marked vitamin D deficiency in patients with diabetes in the UK: ethnic and seasonal differences and an association with dyslipidaemia. *Diabet Med.* 2012;29:1343–5.
 163. Lee JI, Oh SJ, Ha WC, Kwon HS, Sohn TS, Son HS, Cha BY. Serum 25-hydroxyvitamin D concentration and arterial stiffness among type 2 diabetes. *Diabetes Res Clin Pract.* 2012;95:42–7.
 164. Nykjaer A, Dragun D, Walther D, Vorum H, Jacobsen C, Herz J, Melsen F, Christensen EI, Willnow TE. An endocytic pathway essential for renal uptake and activation of the steroid 25-(OH) vitamin D3. *Cell.* 1999;96:507–15.
 165. Anderson RL, Ternes SB, Strand KA, Rowling MJ. Vitamin D homeostasis is compromised due to increased urinary excretion of the 25-hydroxycholecalciferol-vitamin D-binding protein complex in the Zucker diabetic fatty rat. *Am J Physiol Endocrinol Metab.* 2010;299:E959–67.
 166. Swinburn B, Sacks G, Ravussin E. Increased food energy supply is more than sufficient to explain the US epidemic of obesity. *Am J Clin Nutr.* 2009;90:1453–6.
 167. Wanders AJ, van den Borne J, de Graaf C, Hulshof T, Jonathan MC, Kristensen M, Mars M, Schols HA, Feskens EJM. Effects of dietary fibre on subjective appetite, energy intake and body weight: a systematic review of randomized controlled trials. *Obes Rev.* 2011;12:724–39.
 168. Du H, van der A DL, Boshuizen HC, Forouhi NG, Wareham NJ, Halkjaer J, Tjonneland A, Overvad K, Jakobsen MU, Boeing H, et al. Dietary fiber and subsequent changes in body weight and waist circumference in European men and women. *Am J Clin Nutr.* 2010;91:329–36.
 169. Slavin JL. Dietary fiber and body weight. *Nutrition.* 2005;21:411–8.
 170. Aziz AA, Kenney LS, Goulet B, Abdel-Aal ES. Dietary starch type affects body weight and glycemic control in freely fed but not energy-restricted obese rats. *J Nutr.* 2009;139:1881–9.
 171. Keenan MJ, Zhou J, McCutcheon KL, Raggio AM, Bateman HG, Todd E, Jones CK, Tulley RT, Melton S, Martin RJ, et al. Effects of resistant starch, a non-digestible fermentable fiber, on reducing body fat. *Obesity (Silver Spring).* 2006;14:1523–34.
 172. Zhou J, Martin RJ, Tulley RT, Raggio AM, McCutcheon KL, Shen L, Danna SC, Tripathy S, Hegsted M, Keenan MJ. Dietary resistant starch upregulates total GLP-1 and PYY in a sustained day-long manner through fermentation in rodents. *Am J Physiol Endocrinol Metab.* 2008;295:E1160–6.
 173. Rolls BJ, Roe LS, Meengs JS. Salad and satiety: energy density and portion size of a first-course salad affect energy intake at lunch. *J Am Diet Assoc.* 2004;104:1570–6.
 174. Yao M, Roberts SB. Dietary energy density and weight regulation. *Nutr Rev.* 2001;59:247–58.
 175. Rolls BJ, Bell EA, Thorwart ML. Water incorporated into a food but not served with a food decreases energy intake in lean women. *Am J Clin Nutr.* 1999;70:448–55.
 176. Blatt AD, Williams RA, Roe LS, Rolls BJ. Effects of energy content and energy density of pre-portioned entrees on energy intake. *Obesity (Silver Spring).* 2012;20:2010–8.
 177. Willis HJ, Eldridge AL, Beiselgel J, Thomas W, Slavin JL. Greater satiety response with resistant starch and corn bran in human subjects. *Nutr Res.* 2009;29:100–5.
 178. Bodinham CL, Frost GS, Robertson MD. Acute ingestion of resistant starch reduces food intake in healthy adults. *Br J Nutr.* 2010;103:917–22.
 179. Klosterbuer AS, Thomas W, Slavin JL. Resistant starch and pullulan reduce postprandial glucose, insulin, and GLP-1, but have no effect on satiety in healthy humans. *J Agric Food Chem.* 2012;60:11928–34.
 180. Tapsell LC. Diet and metabolic syndrome: where does resistant starch fit in? *J AOAC Int.* 2004;87:756–60.
 181. Ranganathan S, Champ M, Pechard C, Blanchard P, Nguyen M, Colonna P, Krempf M. Comparative-study of the acute effects of resistant starch and dietary-fibers on metabolic indexes in men. *Am J Clin Nutr.* 1994;59:879–83.
 182. Howe JC, Rumlper WV, Behall KM. Dietary starch composition and level of energy intake alter nutrient oxidation in "carbohydrate-sensitive" men. *J Nutr.* 1996;126:2120–9.